Quality of bone healing: Perspectives and assessment techniques

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ABSTRACT

Bone regeneration and healing is an area of extensive research providing an ever-expanding set of not only therapeutic solutions for surgeons but also diagnostic tools. Multiple factors such as an ideal graft, the appropriate biochemical and mechanical wound environment, and viable cell populations are essential components in promoting healing. While bony tissue performs many functions, critical is mechanical strength, followed closely by structure. Many tools are available to evaluate bone quality in terms of quantity, structure, and strength; the purpose of this article is to identify the factors that can be evaluated and the advantages and disadvantages of each in assessing the quality of bone healing in both preclinical research and clinical settings.

Bone is best understood from a bioengineering perspective as a composite with hierarchical organization and from a biological perspective as a connective tissue specialized for load bearing. Embryologically, the formation of bone occurs via two routes: intramembranous and endochondral ossification.[1] Intramembranous ossification occurs by direct ossification in regions of high cellularity on an organized matrix, best observed in the flat bones such as the calvaria, clavicle, and mandible. Endochondral ossification is characterized by a distinct intermediate cartilage which calcifies and is then remodeled. Interestingly, fracture repair involves both endochondral, around the central region, and intramembraneous more peripherally adjacent the corticies and periosteum. The molecular pathways of bone healing appear to recapitulate embryonic skeletal development.[2]

The organization of bone is primarily suited to load bearing with two distinct configurations: an inner, porous, cancellous architecture and an outer, denser, cortical bone (Figure 1). Dense cortical bone comprises around 80% of skeletal mass; the remaining 20% cancellous comprises greater than 60% of total bone surfaces. Though surface-to-volume ratios are eight times greater in cancellous than cortical, the process of remodeling is essentially identical in each.[3] Bone is uniquely restricted to appositional growth; therefore, all activities occur on bone surfaces, either the outer periosteal or marrow-oriented endosteal surface.[4] Bone growth, modeling, occurs during growth and in adults to sculpt shape in response to mechanical loads (mechanical adaptation). Bones are constantly renewed by remodeling to achieve or maintain biomechanically and metabolically competent bone, preserving bone strength by replacing fatigued bone with mechanically sound new bone.[5] Woven bone remodels to lamellar bone and old fatigued remodels to new lamellar. Cortical thickness decreases with age which is also accompanied by a gradual thinning of trabecular plates.[6]

Bony deficits or instability resulting from trauma or disease require intervention to prevent patient disability. By the year 2020, over 60 million people will be at risk for fractures due to osteoporosis or low bone mass.[7] The term "bone quality" has historically been associated with the clinical assessment of fracture risk to indicate that it encompasses more than just bone mineral density (BMD). Specifically, it has been suggested that bone quality is an overall descriptor of bone mass, bone geometry, and tissue material properties that together contribute to overall bone strength.[8,9]

More recently, craniomaxillofacial (CMF) operations for the correction of bony defects[10] along with serious battle injuries involving CMF[11–13] and extremity fractures[14,15] place an additional clinical burden for reconstruction of bony defects. The presence of compromised healing environments further increases these demands.[16] With increasing efforts to develop tissue engineering and regenerative medicine approaches to restore these bone defects, there is a renewed focus on ensuring recovery in the quality of the regenerated bone. The purpose of this article is to delineate metrics for determining the quality of bone healing and review the factors that must be considered and the tools available to do so.

METRICS FOR EVALUATION

In order to define functionally restored bone, it is important to first delineate the multiple functions served by the human skeleton and then evaluate techniques for clinical assessment.

Mechanical load bearing and transduction

The primary functionality of bone is ability to carry load and allow weight bearing, as well as adaptability to changing requirements such as exercise and disuse. Additionally, bone

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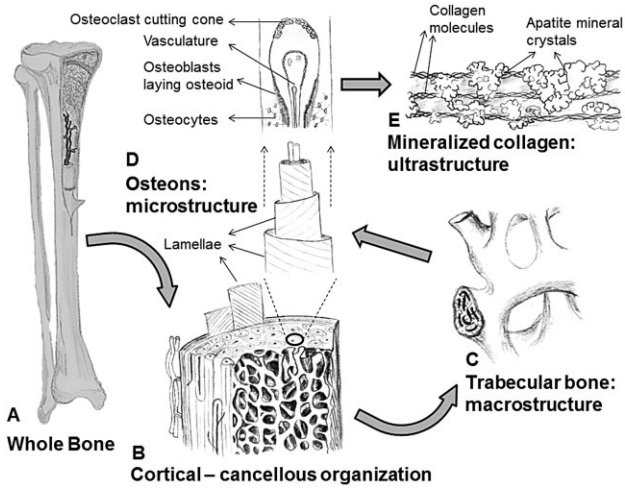

Figure 1. The hierarchical organization of bone: (A) bones can be long or flat to meet physiological functional demands and are comprised of (B) a cortical and (C) a cancellous (trabecular) component. Based on formation and remodeling, (RD) the base functional unit of bone is an osteon with a central Haversian system for blood supply. At the ultrastructural level, (E) bone is a true composite of calcium phosphate mineralized on collagen fibrils.

provides attachment locations to stabilize other connective tissues such as tendons, ligaments, meniscus, and cartilage. From a clinical perspective, bone serves as the anchor for a variety of implants, from nails and screws to dental prostheses and total joint replacements.

Direct mechanical testing

The true efficacy of bone regeneration can be evaluated by the measurement of its mechanical competence in terms of recovery of strength and function. This would be performed by observing relative motion across the bone healing site upon force application. Clinically, the measurement of fracture site stiffness or functional torque via strain gauges or force transducers has been used as an indicator to assess when sufficient stability has been achieved for fixation removal.[17] However, direct biomechanical testing has limited clinical feasibility due to obvious patient discomfort and the necessary removal of fixators.[18] Often, qualitative range of motion and recovery of function scores are also utilized, and in the cases of long bones in the extremities, it may be clinically possible to assess force generation during appropriate movements as a form of mechanical testing.[19] Indirect evaluation of the site can also be performed by using radiography in conjunction with force application to assess relative motion.[20]

Vibrational analysis

Vibrational analysis is based on the propagation of mechanical waves through the tissue and the attenuation in either velocity or amplitude across the healing site. Primary clinical techniques include resonant frequency analysis and ultrasound, while some methods such as computerized sonometry and acoustic emission are more investigational. Compared with mechanical testing, it has the advantage of measuring bone mechanical properties (based on the propagation of mechanical waves) without being invasive.[18] However, these techniques are greatly affected by the interposed skin and soft tissue.[21,22]

Resonant frequency analysis is based on the changes in the frequency at which bone vibrates as healing progresses. A variation of this method is the impulse response method which has been reported to show a greater sensitivity to changes in bending rigidity at earlier time points in healing.[23] It has been shown to be effective in the case of subcutaneous bones[24] but unreliable in the assessment of fracture healing when rigid fixation techniques are used.[25] Ultrasound is another technique that has been utilized clinically and showed a 24% decrease from intact to fractured bone which reduced over time with fracture healing.[26] It has been suggested that the changes observed in transmission speed of sound are primarily due to a change in bone mineralization.[27] The advantages of ultrasound include low cost, ease of use, portability, and a lack of ionizing radiation.[28] Pulsed mode ultrasound has been widely investigated more as a therapeutic technique to accelerate healing of bone fractures.[29,30] It has also been evaluated to assess callus thickness and healing over a range of frequencies,[31] but further standardization of this mode is necessary prior to clinical applications.

Computerized sonometry is based on the transmission of sound across fracture gaps and has been investigated to a limited extent for clinical applications because of high sensitivity to differences in technique, surrounding tissue variation and operator dependence.[32,33] Acoustic emission testing for strength is commonly used for structural testing in mechanical engineering and has been reported to show good results for evaluation of fracture stability to determine timing of fixation removal.[34] Vibrational analysis in general shows high variability which limits clinical application, but this can be addressed to some extent if the techniques are used in comparison with contralateral intact controls instead of in absolute terms.[35]

Shape and form

Although the aesthetics of bone is not as critical in reconstruction as skin, its form houses internal organs such as the brain (skull) and the heart and lungs (rib cage) as well as provides protected passage of vessels and nerves (e.g., vertebrae allow for the passage of the spinal cord and associated nerves, the mandibular foramen allows for the passage of the inferior alveolar artery and nerve). Bone tissue also acts as a space maintainer and provides smooth surfaces in certain locations for ligaments and tendons to slide along and musculature to perform lever functions for movement of limbs. Cancellous bone also houses marrow adipose tissue and hematopoietic precursors which, in addition to being the first responders in the inflammatory reaction that follows injury, produces differentiated red blood cells, white blood cells, and osteoclastic precursors. In the CMF skeleton in particular, the bony skeletal platform, together with the soft tissue, defines facial aesthetics and proportions.

Radiography

The quality of regenerated bone is usually evaluated clinically with radiography (traditional radiography, dual energy x-ray

absorptiometry [DEXA], peripheral quantitative computed tomography [CT]) because it is a noninvasive and nondestructive method. Conventional radiography is the most commonly used method by surgeons for evaluation of bone healing because it is easily available, quick to obtain, and inexpensive. Radiographic images can be scored based on presence of features such as callus shape as well as densification of the regenerate bone to determine healing progression.[36] However, drawbacks of the technique include a disparity among observers on the strength assessment of bone healing from individual radiographs[37] as well as difficulty in assessment when there are multiple surrounding structures such as in the maxillary sinus.[38] Standardized scoring systems such as the radiographic union score for tibial fractures[39] allow for a reduction in the variability of assessing fracture-healing end points[40] and need to be further developed for other sites. Methods for relatively quick quantification of images are also being developed[41] to reduce subjectivity.

DEXA is the tool of choice for osteoporosis management and bone fragility assessment. Photodensitometry,[42] single-photon absorptiometry, and dual-photon absorptiometry[43,44] have also been used to measure bone mineral content to assess fracture risk. These methods are all based on the estimate of BMD by absorption rates of directed photons. Though widely used as an early indicator of fracture risk,[45] DEXA is not three-dimensional and has a limited value as compared with CT measures which include parameters for trabecular architecture and cortical thickness.[46] It is however the tool of choice for osteoporosis treatment and bone fragility assessment. BMD measured by DEXA is reported as a T score which is the difference in the bone density of the individual in standard deviation units from a healthy young population. A T score ≥ -1 is considered normal, between -1 and -2.5 is symptomatic of osteopenia, and $T \leq -2.5$ is indicative of osteoporosis.[47] Similar to T scores, Z scores are differences in individual density from an age-matched population.[48] Other indicators of fracture risk assessment such as age, gender, smoking, alcohol use, prior fractures or family history, and use of drugs such as glucocorticoids should also be considered in conjunction with BMD measures for a better metric.[49] More specific scoring systems such as hip structural analysis and trabecular bone score have also been applied to DEXA images to better indicate bone strength, though they have limited applicability and availability.[50] The limitation of DEXA in dependably predicting fractures stemming from poor bone quality[51] has been one of the primary drivers for the development of better analytical tools that can account for more geometric and material features for a more comprehensive technique to determine fracture risk.[8]

CT is based on acquiring multiple x-ray projections around an object which are then mathematically resolved to generate cross-sectional images of the object based on how much the x-rays are attenuated in passing through the object. While it offers the convenience of being a three-dimensional nondestructive imaging modality, the major drawback is the exposure of the patient to ionizing radiation, many fold higher than conventional radiographs.[52] CT is widely utilized for initial determination of defect size and surgery planning, as well as monitoring healing of skeletal tissues, and has become the technique of choice for bone defect management.[53–55] Quantitative CT allows for image analysis of the dataset to calculate volumetric information from CT data. The major advantage of the technique is the ability to resolve locations with complex structures such as vertebral sites and the maxillary skeleton. The resolution of clinical CT systems ranging between 0.2 and 0.5 mm for high-resolution machines and multislice spiral CTs[56] does not however allow for the most accurate description of trabecular architecture and connectivity. Peripheral quantitative CT is a specialized type of scanner that is optimized for quantitative scans of the extremities, typically the distal radius which allows for high resolution and determination of the cortical and trabecular fractions separately in addition to bone mineral content evaluation.[57]

Magnetic resonance imaging (MRI) is another nondestructive imaging modalities used in the evaluation of bone quality.[58] Although MRI shows good correlation to CT in clinical measures,[59] care has to be taken if patients have metallic fixators or cardiac assist devices such as pacemakers. While high-resolution MRI has been shown to determine trabecular structure to a certain extent, it has also been noted that these estimates are highly sensitive to image postprocessing,[60] suggesting a greater possibility of errors without highly standardized protocols. MRI is however the method of choice to image bone fluid flow and permeability in vivo[61] which is a strong indicator of a successful bone healing response. MRI is also extremely valuable at detecting bone bruises or microtrabecular fractures such as those occurring in joint or spine injuries, which cannot easily be detected by radiographic methods.[62–64] It has also been used in the management of stress fractures in athletes.[65]

The primary advantage of the image-based techniques to determine bone structure and quality are that they are relatively widely available and allow for monitoring bone defects over a time course. The relative advantages and limitations of these techniques have been reviewed in depth by Genant et al.[66]

CALCIUM HOMEOSTASIS

Bone is the largest reservoir of calcium, an important ion in the regulation of multiple cell and tissue processes in the body. Hence, one of the important functions of bone is calcium homeostasis[67] which is tightly regulated by parathyroid hormone[68] and vitamin D.[69]

Both DEXA[70] and photon absorptiometry have been suggested as a method to assess calcium-to-phosphate (Ca/P) ratio in bone in a clinical setting. For example, using photon absorptiometry, the Ca/P ratio in normal adult women is 1.71; it was found to be 1.29 in osteoporotic women,[71] suggesting the value of the Ca/P ratio as a quantifiable metric. A more recent method developed and being tested to evaluate Ca/P ratio involves neutron activation analysis which, while promising at detecting differences,[72] has great clinical safety concerns as it involves the use of gamma radiation. However, a major limitation of Ca/P ratio measurement is the high sensitivity to bone fat content[73] which leads to inaccuracies, and hence, suitable sites must be chosen.

Biological markers for bone healing have also been investigated; however, no single serum marker has been shown to accurately predict fracture union from the limited number of clinical studies on this subject.[26] Various clinical studies have proposed monitoring changes in bone turnover markers from baseline values within 4 hours of fracture incidence,[74] elevated levels of procollagen type III N-terminal peptide indicating a lack of fracture healing completion,[75] and a high correlation between biomarkers and quantitative ultrasound

measurements.[76] Before biological markers can be used clinically to accurately identify the stage of fracture healing and bone stability, many more controlled studies and validation with other imaging modalities are required.

TECHNIQUES OF ASSESSMENT: PRECLINICAL TECHNIQUES

For preclinical models of bone regeneration and repair, there are multiple modalities available to assess the quantity, quality, texture, morphology, and return of function.[58] The advantages and disadvantages of some of the popular methods of bone healing measurement are listed in Table 1.

Mechanical testing

Just as bone is a hierarchically organized biocomposite, its mechanical properties can also be evaluated at every stage of structural organization. Whole bone testing can be performed in physiologically appropriate loading modes to evaluate return of function: three-point bending or four-point bending to evaluate the flexural properties of the femur,[77] torsional strength of radius–ulna complex,[78] and estimation of the compressive properties of the vertebral body.[79] In terms of bone repair, it is critical to have appropriate controls for normal strength in age, gender, and weight matched animals to estimate the target value for fully healed bone. Cores of cortical or trabecular bone can similarly be used to measure mechanical competence of bone.[80] Fatigue testing under strain control is often used to evaluate mechanical properties of bone grafts and implants as that is the most physiologically relevant loading condition experienced in vivo.[81] The major drawback of this type of testing is the high variability between biological specimens.[82] The biomechanical testing techniques used for the evaluation of preclinical specimen ex vivo have been extensively reviewed by Athanasiou et al.[83]

Microindentation is a type of hardness testing based on measuring the material resistance to a fixed applied load for a known duration and has been investigated for local bone property measurement at the millimeter scale.[84] More recently, reference point indentation techniques have been developed that allow in vivo measurement of bone properties based on microindentation, and promising initial results of distinguishing between patients with normal vs. poor bone quality have been reported.[85,86] These techniques are still investigational for clinical use. Nanoindentation techniques measure similar properties of bone on a submicron scale[87] and can be used on either tissue samples or histological slides. Nanoindentation has been used to evaluate the nanomechanical properties of the regenerating bone callus[88] as well as the local tissue mechanical response around implants.[89] These techniques have been incorporated into computational models of callus formation and bone healing to tie together histology, micro-CT imaging, and experimental mechanical data.[87,90] One of the major drawbacks of the nanoindentation method however is the site specificity of properties, making comparison across samples difficult and the high variability in testing and analysis protocols.[91] It is expected that preclinical and ex vivo experimentation will be used with nondestructive imaging modalities such as micro-CT to develop more robust predictive models for bone healing in the near future.

Bone permeability

Fluid transport within bone is not usually estimated in a clinical setting. Recent advances in MRI and positron emission tomography allow for in vivo evaluation with contrast agents and show a 55% reduction in tracer uptake with fracture healing.[61] Fluid transport quantification techniques however need further validation before broader clinical adoption.

Micro-CT

Micro-CT, the high-resolution counterpart of the clinical CT systems, has grown tremendously in recent years. With standardization across the micro-CT platforms, it is now the "gold standard" in the evaluation of bone microarchitecture and morphology.[92] As the method is based on the attenuation of x-rays, it allows for a three-dimensional determination of density, which in turn allows for the architecture of the material to be characterized.[93–95] This is invaluable as it allows for the assessment of BMD as well as correlation to histomorphometric indices (Figure 2A).[96,97] Architectural indices that can be calculated using micro-CT include density-based metrics (bone volume to tissue volume ratio, bone surface to bone volume ratio, and bone surface to tissue volume ratio), trabecular architecture metrics (trabecular number, trabecular thickness, trabecular separation, and trabecular pattern factor), as well as overall architectural descriptors such as structural model index, connectivity density, and degree of anisotropy. With the use of contrast agents, it is also possible to evaluate vasculogenesis in micro-CT.[98] The geometry of trabecular bone as well as that of bone graft substitutes computed from micro-CT[99] can be used to directly evaluate strains observed in mechanical loading or alternately be incorporated into finite element models for prediction of properties such as strength and fracture risk (Figure 2B). While finite element models could potentially allow for accounting of physiological attachments and multiscale architecture in the calculation of strength and are being investigated for potential clinical applications,[100] their major drawback is the need for significant computational resources, the necessity for individual modeling, and a high sensitivity to applied loading conditions. As micro-CT can be used to digitally compute the solid as well as the porous volume of a structure, it has also been used to evaluate the permeability of fluid through biomaterials using computational fluid dynamics to better understand the properties influencing bone in-growth.[101–103] Thus, micro-CT can be used to tie together multiple desired functional outcomes of bone: histomorphometric architecture evaluation, bone mineral quality, and prediction of biomechanical strength.[104] Micro-CT quantification is sensitive to technique settings, such as the selection of appropriate thresholds for distinguishing materials and selection of regions of interest, and will have broader impact with greater standardization,[92] which will allow for direct comparisons across studies. Newer generations of this technology are called nano-CT, and though they allow images with resolution close to 1 μ,[105] they are currently severely limited in terms of sample size and preparation for good image acquisition. Another concern with high-resolution image-based analyses is the size of the datasets and the complexity of calculations which requires significant computational resources and time to process.

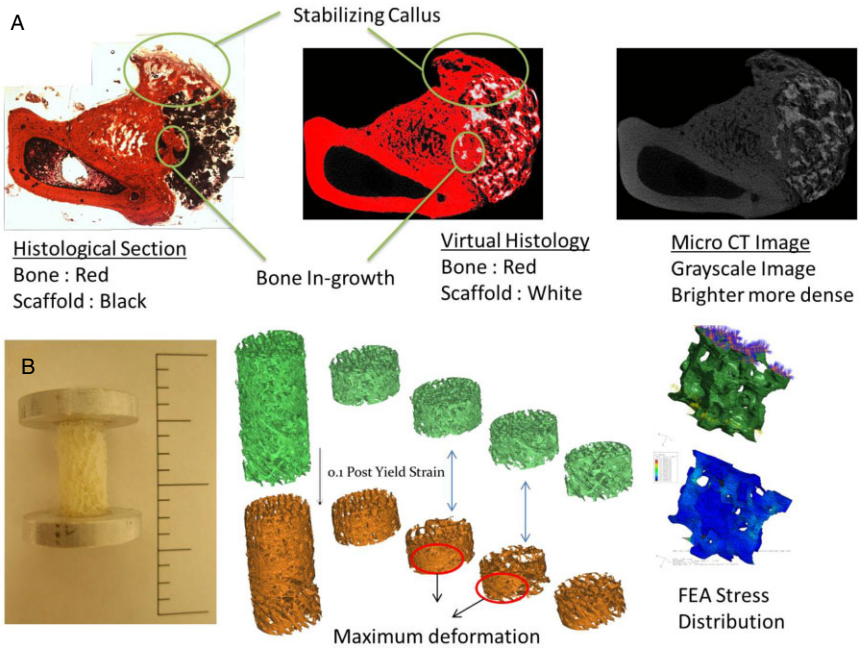

Figure 2. (A) Hydroxyapatite scaffold (stained black) and bone (stained red) in the histological axial cross section are detected based on density and color threshold (bone: red; scaffold: white) from the micro-CT grayscale image. (B) The architecture of a core of human trabecular bone from the femoral neck is evaluated before (green) and after (orange) 10% postyield strain to identify locations of maximum deformation. These architectures can be correlated to strength using finite element models.

Histology

The most direct measure of bone remodeling and assessment of tissue maturity is histology and the quantitative assessment of histological samples (histomorphometry) which allows for a comprehensive analysis of the bone development and remodeling through both static and dynamic indices, as well as an assessment of the microarchitectural features of bone.[106] A standardized set of indices[107] allows for comparison across species, and an extensive set of derived indices allow for the characterization of the complex three-dimensional architecture of trabecular bone to be evaluated. Appropriately stained histological sections can be used to evaluate the callus; blood vessel counts can be used to evaluate vasculogenesis, and polarized light can be used to evaluate collagen bundle organization and the lamellar orientation in osteons. Immunohistochemistry and in situ hybridization techniques can be performed on embedded bone sections to detect osteogenic markers (such as alkaline phosphatase, collagen type I, osteonectin, osteopontin, osteocalcin, and bone sialoprotein) in both cells and matrices to evaluate the stage of bone regeneration in terms of osteogenic differentiation and matrix mineralization and maturity.[108] The use of fluorochrome labels in bone regeneration and tissue engineering research allows for the determination of the onset time and location of osteogenesis and the chronology of bone repair captured on a histological section.[109] While the use of fluorochrome labels allow for some longitudinal observations over the time, it remains an invasive and destructive testing modality that requires either a tissue biopsy clinically or animal sacrifice in preclinical evaluation. The process is also heavily labor and resource intensive.

Tissue compositional analysis

Multiple methods are used to characterize the quality of bone tissue samples. What they share in common is offering specific information on the chemical and material characteristics of bone which has value in better understanding the biochemical processes of normal, healing, aging, and pathological bone. While therapy discovery and design is aided by this information, direct interpretation of these results from a clinical perspective is limited.

Scanning electron microscopy (SEM) can be used in multiple forms to evaluate bone. As the electron beam in SEMs results in the generation of secondary electrons, backscattered electrons, or x-rays depending on the detection method used, the system can be used for high-resolution imaging of surface features, back scattered imaging to generate a tissue mineral density map, or energy dispersive x-ray (EDX) analysis to determine an elemental map of the bone surface.[110] The back scattered energy mode also allows evaluation of the age of osteons based on the maturity of the mineral density laid down,[111] while EDX spectrometry can evaluate Ca/P ratio in bone mineral, as well as the relative amounts of other trace elements.[58,112]

Fourier transform infrared (FTIR) and ***Raman spectroscopy*** measure the relative prevalence of different types of chemical bonds present in bone to evaluate the relative composition of both the collagenous matrix as well as the bone mineral in a two-dimensional spatial fashion.[58] These techniques can also be used to determine mineral-to-matrix ratio, relative collagen cross-links, and mineral size, as well as the extent of carbonate substitutions in the mineral.[113] These metrics can be used to clearly distinguish normal from osteoporotic bone.[114] However, this analysis is largely limited to the benchtop as FTIR requires thin dehydrated specimens, and Raman spectroscopy can be run on thick hydrated specimens. In a promising development, applications of Raman spectra transcutaneously have been recently developed and are being investigated in preclinical models[115,116] at sites with minimal overlying soft tissue for a promising noninvasive technique of determining bone composition.[117]

Table 1. The primary methods for evaluation of healing and the corresponding parameters measured in ex vivo samples or clinical evaluation are listed with their corresponding advantages and disadvantages

Testing methodology	Factor evaluated	Advantage	Disadvantage
Mechanical testing			
Testing to failure	Strength, elastic modulus, toughness	Direct quantitative assessment	Destructive testing
Dynamic testing	Fatigue properties, crack propagation	Survival measurement	Large number of samples
Micro/nanoindentation	Local mechanical properties, hardness	Microarchitectural mechanical properties	Not a direct clinical metric
Computed tomography			
Clinical 64/512 slice CT	Form, architecture, bone mineral density	Nondestructive testing	Low resolution
Contrast enhanced CT	Blood vessel in-growth	Direct correlation to destructive tests	X-ray radiation exposure
Micro/nano-CT	Microarchitecture in 3D	Chronological studies on same specimen	Cost and accessibility
Tissue histology			
Histomorphometry	Bone architecture, cellularity, microstructure	Assessment of tissue type, remodeling	Destructive testing
Polarized light	Collagen organization in osteons, osteoid	Quantitative, highly standardized	Labor intensive, time consuming
Immunohistochemistry	Markers for tissue maturity, ossification stage	Biological relevance, cell staining	Labor intensive
Fluorochrome staining	Bone growth rates, local remodeling rates	Add longitudinal measures to histology	Fluorochrome effect on remodeling
Dual energy x-ray absorptiometry	Bone mineral density, bone mineral content	Nondestructive	Weak correlation to mechanical tests
		Clinical predictor of fracture risk	No separation of cortical, trabecular
Quantitative ultrasound	Combination of density, stiffness, structure	No radiation exposure	No specific correlation to one property
	Measure speed + attenuation of sound in bone	Low cost and ease of operation	Identifies region not specific site
Magnetic resonance imaging	Bone + vasculature, time lapse imaging	Nondestructive	Accessibility, low resolution vs. CT
	Measures permeability, streaming potentials	Provides vasculature information	Concerns if metallic implants, fixators
Mineral/protein composition			
SEM + BSE/EDAX	Mineral density distribution: osteon age (BSE) Ca/P ratio in mineral (EDAX)	Good correlation with nanoindentation	Destructive technique, specialized
FTIR	Mineral–matrix ratio, carbonate substitutions	Spatial mapping of composition possible	Performed on embedded specimen
Raman spectroscopy	Mineral and matrix chemical compositions	Can be done on wet samples	Same drawbacks as histology

BSE, backscattered electron; Ca/P, calcium-to-phosphate; CT, computed tomography; EDAX, energy dispersive x-ray spectroscopy; FTIR, Fourier transform infrared spectroscopy; SEM, scanning electron microscopy.

Nuclear magnetic resonance has been used to analyze the water content and mineral structure of bone.[118] The additional benefit of this technique over traditional MRI techniques is the ability to distinguish between bound water in the matrix and free water in the pore space of bones which might allow for better understanding of the fluid flow in bone tissue.[119] Recent studies have also explored special sequences to detect the bone mineral in in vivo scanning by suppressing the water and fat signal usually seen during magnetic resonance.[120] The technical complexity and equipment needs associated with the process suggest that this technique is quite far from in vivo applicability.

Direct bone material analysis

The alternate method for experimental determination of BMD is direct calculation by calcination of a fixed volume of bone and using its wet and dry weight to calculate the mineral fraction and its density.[118,121] This is called gravimetric analysis, and while it is a relatively simple process, it does not offer spatial resolution as it involves tissue homogenization into a single sample.[122] Similarly, for the protein fraction, collagen in bone tissue can be analyzed to determine total quantity by measuring hydroxyproline concentration after acid dissolution.[123] Additionally, the relative quantities of immature and mature cross-links present can be determined using high-pressure liquid chromatography.[124] Similar to mineral analysis, collagen analysis also does not offer spatial resolution. The component mineral and collagen quality affects the overall mechanical state of bone, and both fractions show distinct changes based on aging or pathological state.

Other techniques from materials science used to evaluate bone properties include scanning acoustic microscopy[125,126] and atomic force microscopy.[127] The different techniques for in vitro and ex vivo evaluation of bone quality have also been reviewed in further detail by Donnelly[8] and Chappard et al.[128] While many of the techniques discussed in this section are actively researched in the laboratory environment and used extensively to characterize material samples and ex vivo preclinical models, they find little application in the in vivo setting.

SUMMARY

The determination of proper metrics for the desired bone healing quality based on site, size and impact of the injury, and the method of treatment and fixation selected is essential. While clinical methods to determine bone quality are based on the historical need to assess fracture healing, new evaluation schemes will likely arise as there is an increase in bone defect regeneration using biomaterial and drug delivery-based systems. Similar to the development of synthetic graft materials, the proper diagnostic modalities for evaluating the requisite benchmarks of bone quality, quantity, strength, and structure can also be appropriately measured and correlated in preclinical research and then employed in clinical translation.

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